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**Introduction**

A central goal of current cancer research is the identification of the genes involved in tumorigenesis, and the definition of the precise role that these genes play in tumor development. Analysis of human breast carcinomas has implicated a number of genes in the genesis of these tumors, including *Erb*B2[43], *HST* and *INT2* [1], *p53* [15], *src* [39], *Rb* [26]. It is suggested by a number of studies that the development of breast cancer in humans requires changes in more than one of these genes, which may in part explain the long latency associated with this disease [15].

*Erb*B2 encodes a receptor tyrosine kinase related to the receptor for epidermal growth factor (EGFR or ErbB), and is amplified in nearly 30% of human cancers, particularly intraductal carcinomas[17, 44] . Numerous studies suggest that this amplification leads to increased mitogenic signaling in the cell. The importance of this amplification is supported by the finding that 70% of transgenic mice that overexpress rat *Erb*B2 in the mammary gland develop mammary carcinomas [11]. However, the latency of tumorigenesis is relatively long (over 200 d), suggesting that other oncogenic events are necessary. Analysis of these tumors revealed small in-frame deletions in the *Erb*B2 transgene in 65% of tumors analyzed [42]. These deletions resided in the extracellular domain adjacent to the transmembrane domain, and resulted in activation of ErbB2 tyrosine kinase activity. These findings indicate that activation of ErbB2 tyrosine kinase activity plays an important role in the development of these tumors. This is consistent with previous experiments, showing that mice carrying an MMTV-driven rat *Erb*B2 transgene with an activating mutation in the transmembrane domain develop multifocal mammary carcinomas with a significantly shorter latency [35].

In 30% of human breast carcinomas, expression of *ERB*B2 is associated with the presence of mutant *p53*, suggesting that activated tyrosine kinase receptors cooperate with mutant p53 in the development of these tumors [15]. p53 is a multifunctional protein that is involved in the regulation of growth of nearly all cell types within mammalian organisms (reviewed in [23]. The wild type p53 protein can suppress tumor cell growth [10], and likely functions as a regulatory protein in two capacities: as a key

component of apoptotis pathways within the cell [46]; and as a checkpoint protein to control G1 to S transition in the presence of genotoxic stress [24]. Structural domains of p53 include an amino-terminal transcriptional activation domain, a central DNA binding domain, and a carboxy-terminal domain important for oligomerization (reviewed in [23]). Genetic alterations at the *p53* locus are common in human cancers, and are primarily either missense mutations or allele loss [2, 14, 36]. While the majority of human tumors with altered *p53* have one allele bearing a missense mutation and one null allele, occasionally tumors are found to have one mutated allele and one normal allele [36]. These findings suggest a progression model in which the initial event is a missense mutation in one p53 allele, leading to a proliferative advantage, and then loss of the other allele, which confers a further selective advantage.

p53 point mutations are highly clustered into four regions that correspond to evolutionarily conserved domains of the protein that function in DNA binding. Some of the most commonly mutated amino acids are those that make direct contact with the DNA [4]. p53 proteins bearing these mutations have been found to have altered DNA binding and transactivation properties [20, 21]. Some mutant proteins fail to activate normal target genes, such as p21, but can activate atypical targets, such as *MDR1* [3]. Thus, certain mutations in p53 may lead to the acquisition of novel and dominant activities within the cell. It is evident from a number of studies that certain missense mutations in p53 function as dominant negative alleles that encode proteins that lack transcriptional activation potential, but retain the ability to oligomerize and thus can pull wildtype p53 into nonfunctional complexes [34]. An example of this is the 135V mutation, which can accelerate tumor development in heterozygous but not nullizygous p53-deficient mice [12]. Other alleles, such as 143A, 175H, 248W, 248Q, 273H, and 281G act as dominant oncogenic alleles, since they can confer new malignant phenotypes upon gene transfer into cells that lack p53 [8, 16]. These phenotypes include the ability to grow in soft agar, and to form invasive tumors in nude mice. The molecular mechanisms that underlie the ability of mutant p53 alleles to induce these changes are unknown.

*p53* alterations are common in human breast carcinomas [5, 38]. Missense mutations have been identified at many of the hotspot regions, including codons 175(R to H), and 248(R to Q). 175H represents approximately 8% of all p53 mutations in human breast cancers. These alleles are dominantly oncogenic in cell culture and nude mouse tumorigenicity assays [8, 16]. To obtain a more accurate picture of the effect that the 175H allele has on mammary cell growth, we used transgenic mice in which this allele was targeted to the mammary epithelium using the whey acidic protein (WAP) promoter. It was somewhat surprising to find that, despite high level expression in the mammary gland, mice carrying the WAP-driven p53-175H were not abnormally susceptible to mammary carcinomas – only one mouse developed a mammary carcinoma and this was with a latency of 11 months [27]. These data suggested that this allele is not dominantly oncogenic on its own in this setting, and requires other cooperating events. Indeed, these mice were much more susceptible than nontransgenic control mice to mammary tumors induced by carcinogens that are known to activate Ha-Ras [25, 28, 32]. This suggests that activated Ras is one molecule that can cooperate with p53-172H in this system.

It is known that ErbB2 can initiate a mitogenic signal within the cell, and that this signal utilizes the same pathway as activated ras. This suggested that if p53-172H can cooperate with activated Ras, it may also cooperate with ErbB2. In our previous studies, we demonstrated cooperativity between ErbB2 and p53-172H in the development of mammary carcinomas [29]. These bitransgenic mice constitute a model system that closely mimics the genetic changes in human breast cancers, and that allows for further studies to uncover the mechanism of cooperativity between these two genes.

**Body**

***Experimental Methods***

**Transgenic Mice** The *p53-172H* transgenic mice, in which mutant *p53* transgene was preferentially overexpressed in the mammary epithelium by use of the whey acidic protein (WAP) promoter, were created and characterized as described [27]. p53 knockout mice were obtained from Tyler Jacks [18]. Unactivated *Erb*B2 transgenic mice (line N#202) which contain the wild type rat *Erb*B2 gene driven by MMTV have been described previously [11]. All three lines are on an FVB background. *p53/Erb*B2 bitransgenic mice were generated by crossing female and male offspring of line 8512 *WAP-p53-172H* transgenic mice to offspring of line N#202 of *MMTV-Erb*B2 transgenic mice. Mouse tail DNA from the offspring of this cross was isolated as described previously [29]. The WAP-172H and/or *MMTV-Erb*B2 transgenes, and the p53 knockout allele were identified by PCR as described [18, 29].

**Histologic and immunohistologic analysis** Mammary glands and mammary tumors were surgically removed, fixed in 10% neutral buffered formalin (ANATECH LTD, Battle Creek, MI) for 6 h, and placed in 70% ethanol until processed. These tissues were embedded in paraffin, and 5 _m sections were placed on regular slides and stained with hematoxalyn and eosin. Immunoperoxidase was performed on deparaffinized tissue sections using standard techniques. To detect vascular endothelial cells, an anti-CD34 antiserum was used (provided by D. Krause, Yale University). To detect phosphorylated neu protein, an antibody directed against a phosphopeptide within the cytoplasmic domain of activated Neu was used (courtesy of M. DiGiovanna).

**Spectral Karyotyping.** In situ hybridization of fluorescently labeled chromosome-specific probes was performed by Dr. Alan Coleman of the NIH, using techniques described by T. Reid and coworkers [41]

***Results and Discussion***

**1. Assessing the genetic function of the 172H allele: dominant negative or dominant oncogenic.**

Our previous work describes the creation of a mouse mammary tumor model in which two of the most frequent changes in human breast cancers – amplification of *Erb*B2 and a dominant oncogenic mutation of *p53* – have been recapitulated. This model serves to address two important issues in tumor development: the mechanism of cooperation of genes in mammary tumorigenesis, and the effect of dominant oncogenic alleles of *p53* on tumor growth in an in vivo experimental model.

It is known that dominant oncogenic mutants of p53 such as 175H can cause immortalization of primary cells [40], can cooperate with Ras in transforming primary cells [9, 13], and can enhance the tumorigenic potential of cells lacking p53 [8]. 175H is particularly potent, being able to induce growth of SAOS-2 cells in agar, where other mutant alleles are not [8]. The rapid kinetics and high efficiency of cooperation in these assays by dominant oncogenic alleles of p53 indicate a direct effect on tumor cell growth. That these effects can be seen in the absence of endogenous p53 argues that these alleles are not acting simply as dominant negative alleles, by inactivating wildtype p53 function. These features of cellular transformation mediated by mutant p53 alleles suggest that these alleles act not only by interfering with p53-dependent functions such as apoptosis, senescence, or genomic instability, [all of which have been suggested as important tumor-promoting sequelae of p53 loss [19, 31, 33, 45]], but also by exerting a dominant effect on cell growth. The nature of this effect is unknown.

We attempted to determine if the 172H p53 allele had the capacity to set in a dominant oncogenic manner. We have had difficulty concluding if this is the case. One would predict that a dominant oncogenic allele of p53 would accelerate tumorigenesis even in the absence of endogenous p53. We crossed the p53172H allele together with MMTV-Neu onto a p53-/- background wishing to compare the latency of mammary tumorigenesis to that seen with: a) 172H and MMTV-neu on a p53 wt background; b) MMTV-neu on a p53-/- background without 172H. Unfortunately, the spectrum of tumors and latency seen in all p53-/- mice was the same (lymphomas and sarcomas) regardless of what other transgenes were present.

**Table 1.**

| group | genotype | latency | % MT | %w/sarcoma | %w/lym | #of animals |
|---|---|---|---|---|---|---|
| | | | | | | |
| 9 | p53-/- | 98 | 0 | 57 | 50 | 14 |
| 10 | p53-/-+neu | 92.5 | 0 | 60 | 60 | 10 |
| 11 | p53-/-+172H | 99.5 | 0 | 75 | 58 | 12 |
| 12 | p53-/-+172H+neu | 96.5 | 0 | 50 | 25 | 4 |

The results from the p53-/-mice (groups 9,10.11,12) were uninformative concerning the contribution of the 172H transgene to mammary tumorigenesis on a p53 -/- background. We then examined the incidence and latency of mammary tumors on mice heterozygous of endogenous p53 (+/-), bearing either one, both, or neither of the two transgenes (Table 2).

**Table 2. Mammary Tumor Incidence and Latency in p53 +/- Mice**

| (days) | | #necropsied | %w/MT* | Latency to MT* |
|---|---|---|---|---|
| 5 | p53+/- | 4 | 50% | 296 |
| 6 | p53+/- +neu | 10 | 100% | 245 |
| 7 | p53+/- +172H | 13 | 38% | 300 |
| 8 | p53+/- +neu +172H | 9 | 100% | 237 |

* MT denotes mammary tumor.

The preliminary data from the experiment suggests that in this setting, 172H is not having a significant input, one either the latency or incidence of mammary tumors, with p53+/- genotype, with or without neu.

**Table 3.Latency to Mammary Tumor in p53 +/- Mice (in days)**

| | gp8 | gp6 | |
|---|---|---|---|
| | 172H + neu | neu alone | |
| | 159 | 181 | |
| | 159 | 221 | |
| | 215 | 230 | |
| | 270 | 233 | |
| median = | 229 | 244 | median = 245 d |
| | 246 | 246 | |
| | 260 | 265 | |
| | 265 | 304 | |
| | 277 | 373 | |
| | | 415 | |

While statistical analysis has not been done, it appears that the latency to tumorigenesis is shorter when the 172H transgene is present. However, it is clear that the affect of the 172H allele on the latency of mammary tumorigenesis is not as dramatic in this setting (p53+/- mice) as it was on the p53+/+ background. One concern here is the level of activity of the WAP promoter driving the 172H allele. The WAP promoter is dependent on the hormonal state of the mouse. It is active from day 10 of gestation through lactation. All these mice were housed with male mice to maintain them in a pregnant and/or lactating status. However, due to a change in personnel, the age at which the mice were placed in mating may have differed from that in our previous experiments (with the p53 +/+ mice). In our previous analysis which compared tumor latency of neu vs. neu+172H on a p53+/+ background, the median age of mammary tumor development was 230 days for neu+ mice, and 150 days for neu + 172H bitransgenic mice. It is not clear that the change in personnel can fully explain the difference in latency between the two experiments, since the age at mating was not consistently later in the more recent experiments with the p53+/- mice. Mice having both the neu+ and 172H transgenes were on the p53+/+ background are currently being maintained in parallel with similar transgenic mice having the p53+/+ genotype (Table 4).

**Table 4. Mice currently housed with neu and/or 172H on p53+/+ background**

| Genotype to MT | # alive | median age | # necropsied | latency |
|---|---|---|---|---|
| | | | | |
| p53+/+ neu+ | 13 | 218d | 0 | - |
| p53+/+ neu+ 172H | 7 | 197d | 1 | 265 |
| p53+/+ neu+ | 7 | 175d | 10 | 245 |
| p53+/+ neu+172H | 13 | 183d | 9 | 237 |

This more recent cohort of mice (Table 4) should provide pertinent information regarding tumor latency.

To assess the level of 172H transgene expression in these mice, mammary glands and mammary tumors from mice of the various genotypes are being analyzed by Northern blots using a probe for p53. this will provide information about the level of f expression of the 172H transgene, and may provide an explanation for the differences observed between these current experiments and those that we reported on previously (Li et al. 1997).

One curious result is the development of mammary tumors in mice with p53+/- background without the neu transgene. In previous experiments, we found that p53+/+ mice without neu were not significantly susceptible to mammary tumors. This was true for nontransgenic animals as well as 172H transgenic. Our current experiments show that both p53+/- nontransgenic and p53+/- 172H transgenic mice are susceptible to mammary tumors (Table 2). This phenotype is clear with 1721H+,p53+/- mice: of the ten mice that were allowed to age until either lesion development or age>1 year, five developed mammary adenocarcinomas of the MG. One striking finding in these tumors, which had a median latency of 300d, was the presence of abundant fibrovascular stroma surrounding the islands of neoplastic epithelial cells (Figure 1). This histologic feature appears to be specific for this particular genotype, for it has not been seen in any other tumors. This suggests that it is not a nonspecific hormonal effect related to the stage

of these mice in the pregnancy/lactation cycle at which the tumor was harvested. The well-developed stroma seen in the p53+/-, 172H+ tumors is reminiscent of that seen in human breast tumors, a feature that imparts a scirrous quality to breast tumors. The etiology of this intense stromal reaction in unknown. One attractive possibility is that in the absence of a neu transgene driving the tumor process, tumorigenesis is instead dependent on mutational activation of other oncogenes. We hypothesize that the 172H transgene is assisting tumor development by increasing the frequency of mutational events and genomic instability. This then leads to activation of cooperating oncogenes, which in this setting, may stimulate stromal proliferation. The identity of such an oncogene(s) is unknown but is likely to encode a growth factor (e.g., FGF,PDGF) or angiogenic growth factor (e.g., VEGF or FGF). One of the five p53+/-, 172H+ tumors was explanted to tissue culture, and can thus be analyzed further for growth factor production and karyotypic abnormalities.

**2. Analysis of genomic instability.**

In our bitransgenic model, we do not observe the emergence of tumors with kinetics that indicate direct and immediate malignant transformation by coexpression of 172H and *neu*: tumors arise following the second pregnancy rather than the first, and are unifocal, indicating the necessity for other events. This is thus distinct from the cell culture results described above [8], and is likely due to several things, including the lower transforming potential of native *neu* relative to Ras, the presence of endogenous p53 alleles in our transgenic mice, as well as other tumor control mechanisms that exist in the intact animal, such as tumor immunity, the inhibitory influence of surrounding tissue, and the requirement for tumor angiogenesis. Nonetheless, the 172H allele accelerates *neu*-induced tumorigenesis, albeit by an unknown mechanism. We present several possible mechanisms that our bitransgenic model will allow us to address. These models are based on the known or suggested functions of p53, which include an effect on apoptosis, on genome stability, and on transcriptional regulation of cell growth regulatory genes.

The data from cell culture experiments described above suggest a direct effect of 172H on tumor cell growth, and such an effect may indeed play an important role in our system. However, other effects of this allele are also possible. One is that p53-172H increases the likelihood of additional mutational events in genes other than the *neu* transgene in the nonmalignant cells expressing MMTV-*neu*, and thus accelerates tumor formation. One type of genetic alteration known to contribute to mammary tumorigenesis is gene amplification. While an increased frequency of gene amplification is seen in p53 null cells, it is not observed in Li Fraumeni cells (mutated at 184 or 248) that retain one wild-type p53 gene [30] Since our 172H+*neu* bitransgenic tumors appear by Southern blot analysis to retain (a) wildtype copy or copies of p53 (data not shown), this mechanism may not apply to this model. We are currently assessing the frequency of other types of alterations – e.g., deletions, point mutations – in these bitransgenic tumors.

We are taking three approaches to assessing the contribution of the 172H transgene to mutation frequency and chromosomal instability.

1. Assessing aneuploidy over time in non malignant mammary glands from transgenic and nontransgenic mice.
2. Determination of mutation frequency in mammary tissue of transgenic and nontransgenic mice, using as a mutation sensor a lacI indicator gene residing in a lambda phage transgene.
3. Comparative genome hybridization.
4. Spectral karyotyping.

Approach One: We have harvested mammary glands from normal, 172H+, neu and 172H+neu+mice at various time points, and have processed these for histologic section. H+E slides have been prepared. We will stain these with Feulgen stain, and use image analysis to quantitate the amount of DNA per nucleus.

Approach Two. In collaboration with Dr. Peter Glazer of the Dept. of Therapeutic Radiology at Yale University, we are creating transgenic mice that contain a mutation sensor, which consists of a lambda phage transgene

that contains a lacI gene. Transgenic genomic DNA from these mice can be packaged using standard lambda packaging extracts to yield infectious lambda phage particles. When these are plated on an appropriate indicator strain of E. Coli, one can assess mutation in the lacI gene through blue-white color selection with X-gal. Through this approach, one can determine the mutation frequency in the DNA of any organ within the transgenic mice. We are crossing the lacI transgenic mice with mice containing the 172H and neu alleles to create bi- or tri-transgenic mice that contain lacI. We are now aging these, and will harvest mammary and liver (control) tissues at various time points before and after tumor formation. DNA will be extracted, and will be packaged and assayed for mutation frequency. This experiment will allow us to determine the mutation frequency in nonmalignant and malignant mammary glands in the different genetic backgrounds, which will allow us to assess the influence of the 172H and neu transgenes on mutation frequency.

Approach Three. To document large gains or losses of DNA in tumors arising in mice bearing neu and/or 172H, we are employing comparative genome hybridization (CGH). In this technique, tumor DNA is fluorescently labeled and hybridized to a metaphase spread of normal mouse chromosomes in the presence of normal DNA labeled with a different fluor. Digital photomicroscopy and image analysis are performed to quantitate the relative intensities of the normal and tumor DNA hybridization to the metaphase chromosomes. In this way, regions of DNA overrepresented (amplified) or underrepresented (deleted) in the tumor DNA can be identified. This project is being performed in collaboration with Dr. Alan Coleman at the NIH.

In our lab, we are attempting to perform CGH on nylon filters of mouse BAC libraries instead of on glass slides of metaphase spreads. In this instance, the normal and tumor DNAs are radiolabeled with two different radioactive isotopes ($^{32}P$ and $^{33}P$) that can be distinguished on a Phosphoimager. The advantage of this approach is that the regions of increase or loss are present in the tumor can be translated directly into cloned segments of DNA, which can be further analyzed.

Approach Four. In collaboration with Dr. Alan Coleman at NIH we are also analyzing tumors for chromosomal abnormalities by spectral karyotyping (Figure 2). In this experiment, metaphase spreads are prepared from tumor cells, and these are hybridized with a fluorescent probe mix, which contains a probe for each mouse chromosome labeled with a different Fluor. As a result, each mouse chromosome can be distinguished by the emission spectrum of the fluorescence. With this approach, we have documented a chromosomal translocation in a mammary tumor from a mouse containing 172H and neu on a p53+/- background (Figure 2). We are continuing this analysis to determine if the tumors arising in our mice have consistent nonrandom chromosomal abnormalities. Both the number of abnormalities and the identity of the chromosomal region involved will give insight into the degree of genomic instability in mice of different genetic background, and, potentially, the identity of cooperating oncogenic events.

**3. Documentation of tumor angiogenesis.** An alternative mechanism of *p53*-172H action in this model is that it may promote other aspects of tumor growth, such as tumor angiogenesis. The finding that mutant, but not wild-type, p53 can synergies with PKC to stimulate vascular endothelial growth factor (VEGF) **[22]**, suggests that the 172H allele could stimulate vascular ingrowth, which is known to be a rate-limiting step in tumorigenesis. To investigate this possibility, we are documenting the density of vessels in mammary tissue and tumors of the different genotypes (no transgene, neu alone, 172H alone, and 172H plus neu). This is being done by immunohistochemical staining for CD34, a cell surface protein present on mouse endothelial cells (Figure 3). This staining is highly specific, and allows for straightforward quantitation of microvessel density in mammary glands and tumors of the different genotypes.

**4. Assessment of phosphorylation status of Neu in transgenic tissues, both nonmalignant and malignant.** One key feature of neu-induced mammary tumorigenesis is the marked increase in Neu phosphorylation that appears to accompany tumor formation. One potential mechanism by which 172H could accelerate tumor formation is to hasten this increase in phosphorylated Neu. To document the timing of this increase in tumor progression, we are assessing the presence of phosphoNeu at different time

points leading up to tumor formation, using a anti-phosphoneu monoclonal antibody that has been well characterized by Dr. Michael DiGiovanna [6, 7], with whom we are collaborating. We have been able to obtain specific staining with low background (Figure 3), and are now embarking on the analysis of tissue sections from a panel of nonmalignant and malignant mammary tissue specimens obtained at different ages in our transgenic mice.

**5. Identification of cooperating oncogenes by MMTV proviral tagging.** To further understand the latency of tumorigenesis in our transgenic mice, we have initiated an experiment to identify cooperating oncogenes by MMTV proviral tagging. Two separate experiments are being carried out. In the first, (Table 5), the 172H transgene was put onto a C3H background by backcrossing, and then a transgenic male was crossed to a C3H MMTV+ female mouse, which presumably passed MMTV onto the pups throught the milk (this has yet to be confirmed). We have a cohort of 34 mice with both MMTV and 172H, and 13 mice with MMTV alone.

**Table 5.**

| Genotype | MMTV + 172H | MMTV alone |
|---|---|---|
| Number in Cohort | 34 | 13 |

In a second cross, we are creating 172H/neu bitransgenic mice with MMTV by mating bitransgenic male FVB mice with C3H MMTV+ female mice. This cross generates FVB x C3H F1 hybrid mice, with either one, neither, or both transgenes. To date we have the following numbers in each cohort:

**Table 6.**

| Genotype | Neu −, 172H − | neu −, 172H + | neu +, 172H - | neu +, 172H |
|---|---|---|---|---|
| Number in Cohort | 4 | 3 | 4 | 8 |

As control cohorts, we are also establishing F1 hybrid mice without MMTV, both transgenic and nontransgenic. To date, none of the mice in the MMTV proviral tagging has developed a mammary tumor. We will continue to monitor these mice.

**Conclusions.** We have created a mouse model for human breast cancer closely mimics the genetic changes that occur in the human disease. Twenty-five to 30% of human breast cancers show amplification and overexpression of *Erb*B2 gene, and of these, many will have point mutations in p53 [15]. The 175H mutation is the most common p53 mutation in human breast cancers, and is often accompanied by loss of the other allele, arguing that it is not simply acting as a dominant negative [37]. Thus, we have created a useful model for the study of human breast cancer. We are now investigating the mechanism by which this acceleration occurs, taking a variety of approaches that address the role of genomic instability, tumor angiogenesis, and phosphorylation of Neu. Through these approaches we hope to contribute to the current understanding of mammary tumorigenesis as it occurs in the setting of a whole organism.

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**Figure Legends.**

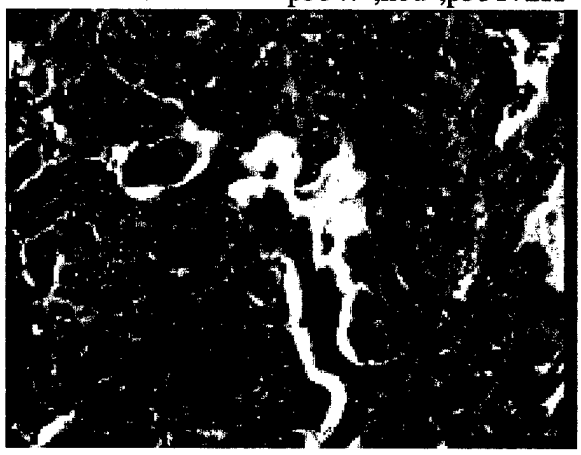
**Fig. 1.** Histology of tumors arising in the mammary glands of mice with various genotypes. Shown are photomicrographs of H+E stained sections of mammary tumors arising in mice with the genotypes indicated to the top of each panel.

**Fig. 2.** Spectral karyotyping of tumor #5132 (p53+/-; neu+; 172H+). Left panel: histopathology (H+E stain); right panel: spectral karyotyping. Arrow denotes translocation involving chromosomes 5 and 7.

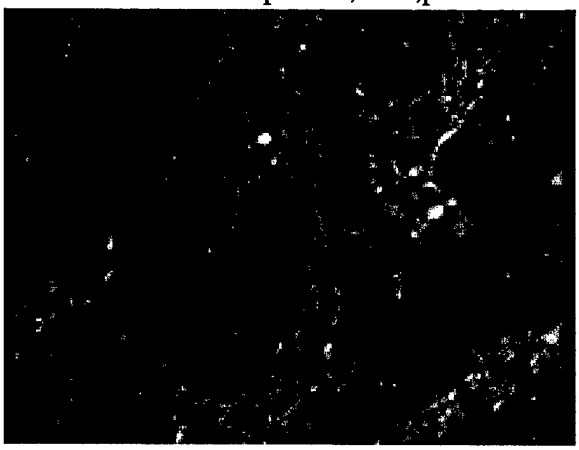
**Fig. 3.** Immunoperoxidase analysis of mammary glands (top panels) and mammary tumors (bottom four panels) performed with the antibodies indicated. Ctl denotes control immunoperoxidase reaction with no primary antibody.

Gr 4 #5545 p53+/+;neu+;p53172H+

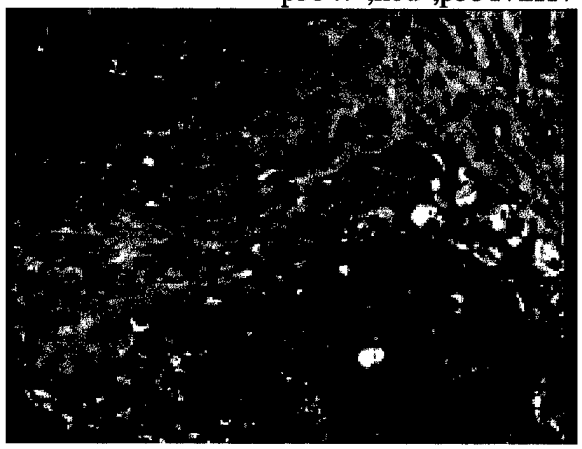
Gr 5 #5035 p53+/-;neu-;p53172H-



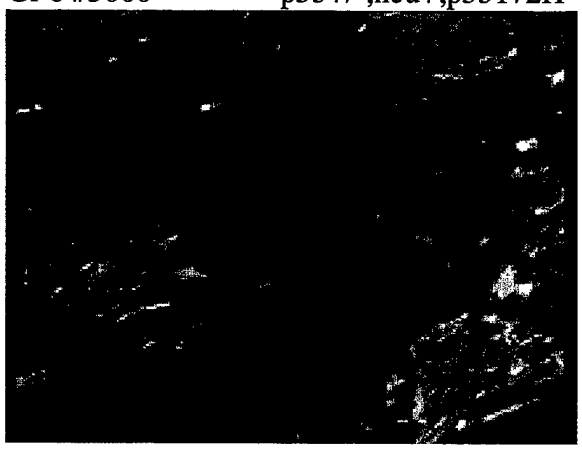
Gr 7 #4923 p53+/-;neu-;p53172H+



Gr 7 #5033 p53+/-;neu-;p53172H+

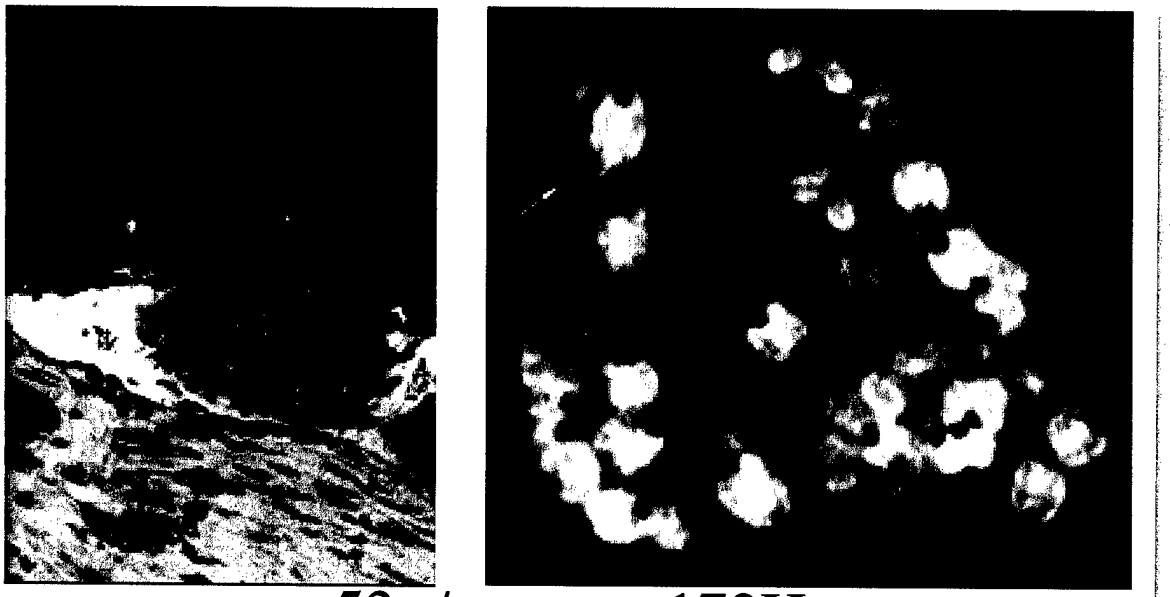


Gr 6 #5066 p53+/-;neu+;p53172H-



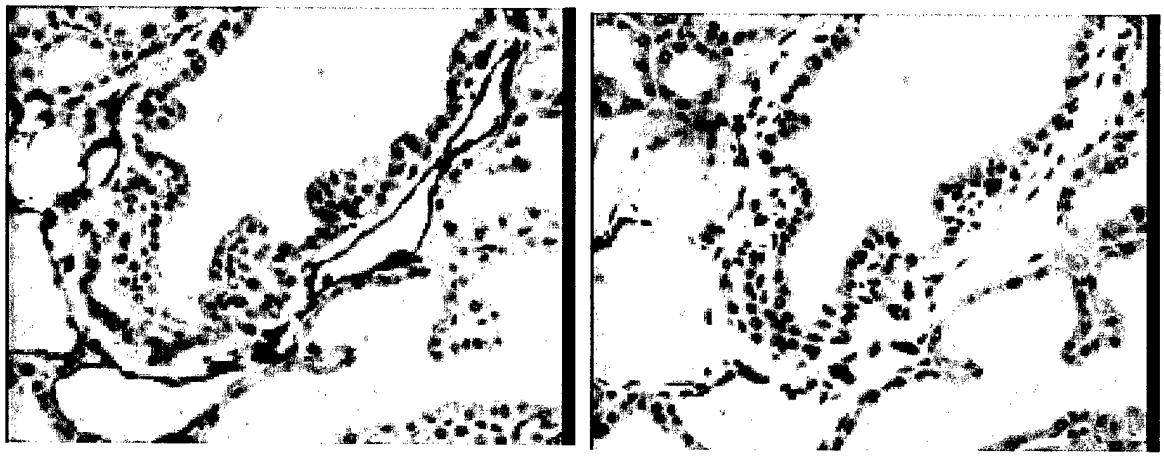
Gr 8 #5132 p53+/-;neu+;p53172H+

Figure 1

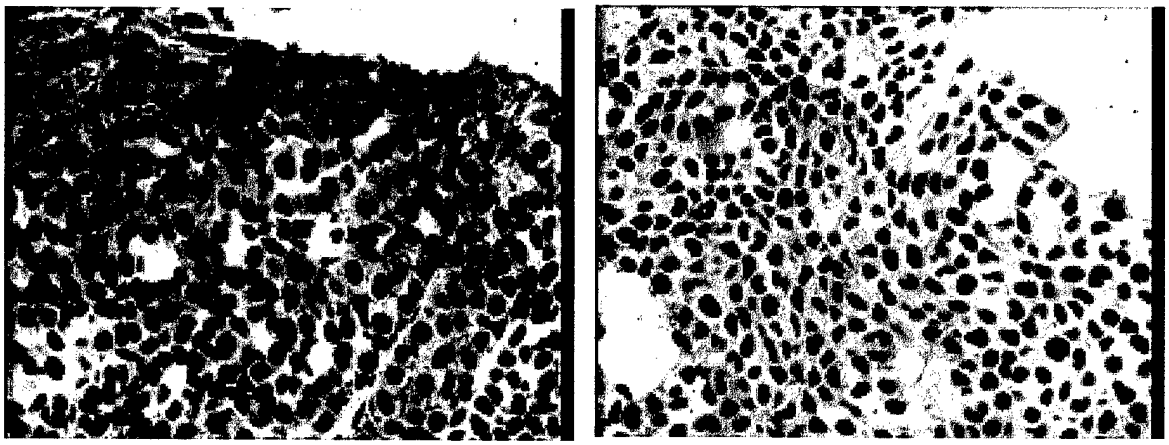


p53+/-; neu+; 172H+

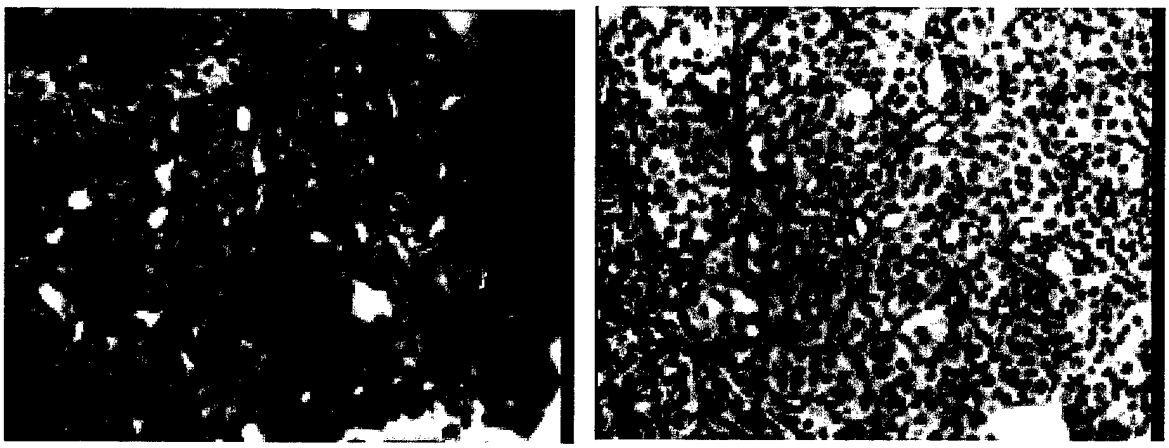
Figure 2



CD34 ImPox Ctl ImPox



P-Neu ImPox Ctl ImPox



P-Neu ImPox Ctl ImPox

Figure 3